Original scientific paper

Adaptive Changes of the Electrophoretic Mobility of Cell Nuclei (EMN) Index in the Intensive Physical Exercise of Male Rowers with Different Training Experience

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ABSTRACT

The aim of this study is to attempt to determine the relationship between the degree of the EMN index and the depth of changes of selected hormones and metabolic parameters as an effect of intensive physical exercise during the training process in male rowers. Juniors (N=62; chronological age 16.4 y.o. SD=1.14 y.; training experience 3.0 y.o. SD=1.05 y.) and seniors (N=27; chronological age 21.4 y. SD=1.73 y.; training experience 5.5 y.o. SD=1.10 y.), in the preparatory period of the training process, performed physical exercise of maximum intensity on a rowing ergometer. Acid-base balance parameters (pH, BE) and the concentration of lactic acid (LA) were determined as the result of physical exercise. Some selected hormones were also indicated (hGH, PrL and Prg) to show their exercise changes. A sample of buccal epithelium cells was taken from each of the male rowers, before and after the exercise, to evaluate the percentage of the EMN index by intracellular microelectrophoresis. A greater differentiation of metabolic changes during exercise was manifested in juniors than seniors. This was reflected in changes of acid-base balance parameters, exercise physiological parameters, hormone concentration and also in changes of the EMN index. These changes were probably dependent on deep metabolic processes of an acid character during exercise. This could prove a more stable homeostasis through more economical metabolic reactions in seniors as the effect of the training process, meaning that seniors were better adapted to heavy physical exercise than juniors.

Key words: *intracellular microelectrophoresis, buccal epithelium cells, EMN index, exercise physiological parameters, male rowers, adaptive changes*

Introduction

The phenomenon of Electrophoretic Mobility of Cell Nuclei (EMN) in buccal epithelium cells shows the status of the organism's homeostasis. The proposed index is dependent on chronological age and can be used as a measure of so-called biological or developmental age[1–4]. Besides this, the EMN index is susceptible to many environmental determinants such as nutrition, risk habits and the intensity of physical activity[5]. The EMN index can also reflect a physiological status. It is known that after exercise a lower mean value of the EMN index is observed and the mobility of cell nuclei in the electric field is slower[6,7]. The aim of the study is to attempt to determine and show similarities in the EMN index and selected physiological parameters changes in male rowers in relation to chronological age and training experience as a result of intensive exercise during the training process.

Materials and Methods

Two groups of male rowers: 62 juniors and 27 seniors were subjected to the study. Training experience was smaller in the junior male group (3.0 y.) as compared to the senior one (5.5 y.). Anthropological measurements ac-

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cording to standard anthropometry procedure[8] and other measurements, including body mass components, were carried out before exercise by the bioanalyzer Spectrum Lightweight R.J.C. System Inc., USA. In the preparatory period of the training process male rowers performed physical exercise of maximum intensity on a rowing ergometer Concept II (Morrisville, USA). Ventilatory physiological parameters were registered continuously during the exercise by a Cardio-Pulmonary-Exercise-D (CPX-D) computer system from Medical Graphics Inc., USA (e.g. oxygen uptake at the level of ventilatory threshold – VO_2VT and maximal oxygen uptake – VO_2max). Heart rate (HR) was determined by a Sport-Tester PE-3000 from Polar Inc., Finland. Power and its variants: power VT, power max and mean power, then time over a distance of 2000 m (time 2000 m), and the numbers of movements on rowing ergometer allowed the determination of mean rate of moves and max rate of moves as registered by the rowing ergometer. Before and after the exercise arterialized blood from the fingertip was drawn and tested to determine the acid-base balance parameters (pH, and basis excess – BE) and the concentration of lactic acid (LA). Before and shortly after the end of exercise venous blood was also drawn and tested from the inside of the elbow, to indicate concentrations of selected hormones: growth hormone (hGH), prolactin (Prl) and progesterone (Prg). Hormones, acid-base balance parameters and values of the EMN index were presented as values in their resting status (rest), exercise status (exerc) and post-pre-exercise difference – including exercise hemoconcentration – (ΔHc). The EMN index was not measured in blood, but in the buccal epithelium cells suspended in saliva and the phenomenon of hemoconcentration was also taken into consideration in the EMN index values after physical exercise to indicate the concentration of all body fluids, with saliva inside the oral cavity included. A sample of buccal epithelium cells was taken from each of the male rowers, before and after the exercise, to evaluate the percentage of the EMN index. To measure this, the intracellular microelectrophoresis – EMN (electrophoretic mobility of cell nuclei) method was used[3-6,9-10]. This method is based on a physicochemical phenomenon – electrophoresis – relating to the mobility of cell structures. The crucial facts are the mobility of cell nuclei in a variable electric field and the change of the proportion between the number of cells with mobile nuclei and cells with immobile nuclei during different phases of ontogenesis and different physiological and metabolic states of the biological system. The percentage value of the EMN index is calculated on the basis of the number of epithelium cells with mobile nuclei in relation to the cells with immobile nuclei per 100 cells counted by the researcher. The analysis was carried out with Statistica 7.1 software using the one-way ANOVA.

The study was approved by the Regional Commission for Research Ethics at the Karol Marcinkowski Medical University in Poznan, Poland.

Results

The examined groups of male rowers: juniors and seniors differed in a statistically significant manner dependent on chronological age and training experience (Table 1). There were no statistically significant differences in anthropometric measurements (Table 1).

A greater differentiation of metabolic changes during exercise of maximum intensity was manifested in juniors as compared with seniors. This was reflected in acid-base balance parameters (Table 2) and exercise physiological parameters (Table 3).

Among acid-base balance parameters of junior and senior male rowers statistically significant differences in LA values were noticed, meaning that there were higher exercise changes for LAexerc and ΔHcLA for seniors (Table 2).

Physiological parameters in the exercise of maximum intensity were better in seniors (Table 3). Results of hormone concentration changes in junior and senior groups under the influence of training process duration (Table 4) reflected also better in the senior group's exercise physiological parameters (Table 3).

Therefore, distinctly higher increases of hormone concentration values in the junior group after physical exercise were observed (Table 4). In the case of hGHexerc, ΔHcPrl and Prgrest statistically significant differences between mean values in studied male rowers were noticed (Table 4). At the end there were also differences between juniors and seniors in changes of the EMN index

TABLE 1
MEAN VALUES OF AGE AND MORPHOLOGICAL FEATURES OF STUDIED GROUPS OF MALE ROWERS

Group	Juniors N=62		Seniors N=27		F	p
Feature	$\overline{X}$	SD	$\overline{X}$	SD		
Chronological age (years)	16.4	1.14	21.4	1.73	22.5173	0.0001*
Training experience (years)	3.0	1.05	5.5	1.10	22.5365	0.0001*
Body height (cm)	185.8	7.60	186.9	6.61	0.3313	0.5664
Body mass (kg))	78.9	7.12	81.0	8.12	1.3233	0.2532
Body mass index BMI (kg/m^2)	22.9	1.87	23.1	1.52	0.4048	0.5263

* $p<0.01$, $\overline{X}$ – mean, SD – standard deviation

TABLE 2
MEAN VALUES OF ACID-BASE BALANCE PARAMETERS IN STUDIED GROUPS OF MALE ROWERS

Group	Juniors N=62		Seniors N=27		F	p
Feature	$\overline{X}$	SD	MEAN	SD		
pHrest	7.3729	0.0170	7.3706	0.0134	0.3213	0.5723
pHexerc	7.1492	0.0751	7.1152	0.0825	3.2314	0.0757
ΔHcpH	–0.2236	0.0712	–0.2554	0.0790	3.1143	0.0811
BErest (mmol/L)	–0.9597	1.1749	–0.5727	1.0584	1.8824	0.1736
BEexerc (mmol/L)	–16.2657	3.8808	–17.7500	4.4807	2.2425	0.1379
ΔHcBE (mmol/L)	–15.3060	3.7693	–17.1773	4.5975	3.6521	0.0593
LArest (mmol/L)	1.3761	0.3555	1.3909	0.3598	0.0285	0.8663
LAexerc (mmol/L)	11.8910	3.5017	14.1136	3.6182	6.5650	0.0121*
ΔHcLA (mmol/L)	10.5149	3.3955	12.7227	3.5013	6.8966	0.0102*

* $p<0.05$, $\overline{X}$ – mean, SD – standard deviation, pH – determination of acidity, BE – basis excess, LA – lactic acid

TABLE 3
EXERCISE MEAN VALUES OF PHYSIOLOGICAL PARAMETERS IN STUDIED GROUPS OF MALE ROWERS

Group	Juniors N=62		Seniors N=27		F	p
Feature	$\overline{X}$	SD	$\overline{X}$	SD		
HRexerc (bpm)	190.7	8.86	185.0	6.24	9.1535	0.0033*
VO_2VT (mL/kg/min.)	41.6	4.85	41.9	8.27	0.0039	0.9505
VO_2max (mL/kg/min.)	55.0	5.72	59.7	6.76	2.9365	0.0990
PowerVT (W)	230.6	38.91	232.1	20.93	0.0080	0.9295
Powermax (W)	346.7	60.21	363.6	28.66	0.4341	0.5160
MeanPower (W)	295.8	41.94	351.2	35.21	22.4047	0.0000**
Time2000m (s)	423.6	18.01	400.2	13.71	22.3466	0.0000**
Mean rate of moves (number)	25.6	3.33	26.5	3.00	1.4483	0.2321
Max rate of moves (number)	30.9	1.62	27.3	1.51	0.2920	0.5935

* $p<0.05$, ** $p<0.01$, $\overline{X}$ – mean, SD – standard deviation, HR – heart rate VO_2VT – oxygen uptake at the level of ventilatory threshold, VO_2max – maximal oxygen uptake

values (Table 5) with greater differentiation in the junior group.

In this report the majority of hormonal levels at rest and, even in their exercise and post-pre-exercise difference (ΔHc) statuses, were lower in seniors than in juniors (Table 4). The one-way ANOVA analysis showed a dependence of the EMN index changes on chronological age and then training experience (differences between junior and senior groups) (Table 5). The electrophoretic mobility of cell nuclei was lower in juniors pre- (EMNrest) and post-exercise (EMNexerc) (Table 5), who were 5.0 years younger than seniors (Table 1) and their training experience was 2.5 years shorter than in seniors (Table 1).

Studied junior and senior groups also showed statistically significant differences in levels of hormone concentration in the case of hGHexerc and ΔHcPrl, statistically higher in juniors, and in the case of Prgrest – statistically lower in this group of subjects.

It can be reasonably concluded that with the noted differences in acid-base balance (Table 2) and exercise physiological parameters (Table 3) in regard to chronological age and training experience of subjects (juniors and seniors), differences in hormone concentrations (Table 4) and the EMN index values (Table 5), as the EMN index – together with hormone changes – can be used as a tool to evaluate physiological changes during physical exercise.

The obtained results show statistically significant differences for the level of the EMN index in resting and exercises statuses between juniors and seniors (Table 5). Between these groups there are differences also in the depth of change between the resting and exercises statuses (ΔHcEMN) averaging a few percent (Table 5). In the case of juniors this difference is more than two times higher.

Discussion and Conclusion

The greater differentiation of metabolic changes during exercise in juniors as compared with seniors was in

TABLE 4
EXERCISE LEVELS OF HORMONE CONCENTRATION IN STUDIED GROUPS OF MALE ROWERS

Group	Juniors N=62		Seniors N=27		F	p
Feature	$\overline{X}$	SD	$\overline{X}$	SD		
hGHrest (mIU/L)	14.39	18.736	11.51	13.339	0.5198	0.4729
hGHexerc (mIU/L)	52.36	41.789	31.36	27.973	5.6886	0.0192*
ΔHchGH (mIU/L)	36.28	42.299	19.14	25.652	3.8054	0.0543
Prlrest (mIU/L)	222.80	208.768	240.26	140.030	0.1520	0.6978
Prlexerc (mIU/L)	360.61	220.872	322.59	129.839	0.6738	0.4143
ΔHcPrl (mIU/L)	131.70	109.261	80.06	68.986	4.9644	0.0288*
Prgrest (nmol/L)	1.87	0.768	2.29	0.800	5.1330	0.0263*
Prgexerc (nmol/L)	2.64	1.049	3.00	0.826	2.3235	0.1316
ΔHcPrg (nmol/L)	0.74	0.565	0.68	0.634	0.1877	0.6660

$^*p<0.05$, $\overline{X}$ – mean, SD – standard deviation, hGH – human growth hormone, Prl – prolactin, Prg – progesterone, ΔHc – it means post-pre-exercise difference in regard to hemoconcentration, then could not be an arithmetic difference between post- and pre- values of a given hormone

TABLE 5
THE EMN INDEX MEAN VALUES IN STUDIED GROUPS OF MALE ROWERS

Group	Juniors N=62		Seniors N=27		F	p
Feature	$\overline{X}$	SD	$\overline{X}$	SD		
EMNrest (%)	36.63	15.438	47.04	12.918	10.9659	0.0000**
EMNexerc (%)	31.46	16.845	44.61	11.598	7.9810	0.0500*
ΔHcEMN (%)	–5.17	12.119	–2.43	14.687	0.2180	0.6417

$^*p<0.05$, $^{**}p<0.01$, $\overline{X}$ – mean, SD – standard deviation, EMN – Electrophoretical Mobilty of Cell Nuclei index

accordance with the effects of sport selection, training experience and chronological age within the range of variability of achieved values of the studied features and parameters[11,22]. This differentiation was confirmed by individual features (Table 1), acid-base balance parameters (Table 2), exercise physiological parameters (Table 3), changes of selected hormone levels (Table 4), and changes in the EMN index values (Table 5). This means that all these elements were involved in the organism's adaptation to cyclic physical exercise.

It was worth mentioning that the differences in the EMN index between these groups were distinctly visible. This fact is important for the usefulness of the EMN index as an indicator of a metabolic status of biological system (Table 5).

The acid-base balance parameters and concentration of lactic acid after exercise in the group of seniors showed more effective changes than in the group of juniors (Table 2). The value of pH was lower at rest and exerc in seniors, but the ΔHcpH was greater than in juniors. In the resting status BE (BErest) was lower in seniors (which was dependent on the greater amount of training), but BEexerc and ΔHcBE were higher and greater than in juniors. Furthermore, the LArest was almost the same in both groups, but LAexerc and ΔHcLA were far higher in seniors than in juniors. This proved the fact that in seniors, with better regulation of blood pH, the possibility of the organism's acidity was greater, and consequently seniors could achieve better exercise results.

The lower exercise changes of the senior group showed more effective, but less costly, changes of their metabolism during exercise. This was proven by all exercise values of physiological parameters – better in seniors than in juniors (Table 3), including lower HRexerc and shorter Time 2000 m (Table 3)[6]. The biological systems of seniors seemed more economic in physical work when comparing them to devices with a determined capacity.

At the end, this was confirmed by values of the EMN index (Table 5)[6,11,12]. From one side, there was a clear influence of chronological age on resting values of the EMN index (EMNrest – Table 5), and from the other – the smaller post-pre-exercise difference of the EMN index (ΔHcEMN) showed the seniors' better economy in performing very heavy exercise of maximum intensity[6,11–12].

A greater mean value of the EMN index was observed during resting status and after physical exercise in seniors than in juniors, which is in accordance with the EMN index properties – in the ontogenesis interpretation – showing a greater mean value for seniors than for juniors (Table 5).

Differences between juniors and seniors are statistically significant (Table 5). Between these groups there are differences also in depth of change between the resting and exercises statuses (ΔHcEMN) averaging a few percent, which are not however statistically significant (Table 5). In the case of juniors this difference is more than two times higher.

This proves the greater level of advancement in chronological age, and thus training experience in seniors. A greater decrease of the EMN index (Table 5) after physical exercise in juniors could prove a greater significance of post-exercise response to exercises stress in juniors. This fact is confirmed by hormonal concentrations in these studies (Table 4).

Accordingly, using the EMN index, as an indicator of biological age and physiological condition of the body[3,6–7,10,23–26], was in agreement with the EMN values in the progressive phase of ontogenesis[1–3], in which, after achieving an optimum point (a maximal value at 17–18 years old), the EMN index values decrease with advancing age[1,3,16] and the whole variability of the EMN index enters into a stable and then involution phase. The chronological age changes in the EMN index were covered by the influence of the metabolic process on acid character, connected with the intensive, competitive training course. The reflection in changes of the acid-base balance parameters, exercise physiological parameters and the studied hormone levels, and also in changes of the EMN index was probably dependent on deep metabolic processes of an acid character. This could prove a more stable homeostasis through more economical metabolic reactions in seniors as the effect of the training process[6,11–12]. Therefore, seniors were better adapted to heavy physical exercise than juniors what had also a connection with a body size of studied rowers. This was in agreement with the greater body height and body mass of finalists compared to nonfinalists of Junior World Rowing Championships in 1997 and 2007[13]. A greater body size of senior versus junior and finalist versus nonfinalist rowers – identified as an important performance related factor in rowing[14,15] – gave then probably better exercise possibilities to perform an intensive exercise. The similar relation between a particular body size and shape of young soccer players and their playing position proved different exercise energy demands and shown their potential in regard to various playing positions[17–22]. There were significant differences between players in body height and body mass linearly related with almost all the power performance variables[16]. This preparation through the training to this position shaped then the specific kind and level of organism's adaptation to a particular, very intensive exercise, as well[16]. It was also manifested in this study.

Both groups of male rowers: juniors and seniors were in the same progressive phase of the EMN index variability during ontogenesis. Only the response of their organisms was different – in seniors – there was a smaller extent of ΔHc hormone post-pre-exercise differences (Table 5) and ΔHc EMN index values (Table 5), which confirmed their better adaptation to the special, competitive training process[6–7,11–12]. On the other hand the organism of seniors can stand deeper acidity (Table 2), giving significantly better exercise physiological results (Table 3).

Generally, the one-way ANOVA analysis showed the influence of the training experience on the depth of selected physiological parameters. It can be concluded that observed changes were probably caused by organism's reaction manifested throughout a decrease of organism's acidity, which was described in our earlier studies and could prove the changes of the EMN index[6,11–12]. In male rowers, as the result of the training process, accordingly to chronological age and training experience, an adaptation to heavy physical exercise increased, which was then reflected by more economical metabolic processes in older, more experienced sportsmen.

Additionally, our present studies showed a more stable homeostasis caused by more economical reactions, in seniors than in juniors (as the effect of training process). This stability was increased through the dependence of the EMN index values on changes of metabolic parameters (pH, BE, LA), exercise physiological parameters and selected hormones. The EMN index can be used as an additional parameter to evaluate the physiological status of the organism during physical exercise and also as a determinant influencing the level of performance during the training process. The EMN index could serve as an indicator of adaptation level to physical exercise in active sportsmen.

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ADAPTIVNE PROMJENE INDEKSA ELEKTROFORETIČKE POKRETLJIVOSTI STANIČNE JEZGRE (EMN) U INTEZNIVNOJ FIZIČKOJ VJEŽBI MUŠKIH VESLAČA S RAZLIČITIM ISKUSTVOM TRENIRANJA

S A Ž E T A K

Cilj ove studije bio je pokušati odrediti odnos između stupnja EMN indeksa i dubine promjene određenih hormona i metaboličkih parametara kao utjecaja na intenzitet fizičke vježbe tijekom procesa treniranja mladih muških veslača. Juniori (N=62, prosječna kronološka dob 16,4 godine, SD=1,14 godine, prosječno iskustvo vježbanja od 3 godine, SD= 1,05 godina) i seniori (N=27, prosječna kronološka dob 21,4 godine, SD=1,73 godine, prosječno iskustvo vježbanja od 5,5 godine, SD=1,10 godina), u periodu pripreme za proces treninga, odradili su fizičku vježbu s maksimalnim intenzitetom na veslačkom ergometru. Parametri ravnoteže na bazi kiseline (pH, BE) i koncentracija mliječne kiseline (LA) određeni su kao rezultat fizičke vježbe. Također, istaknuti su određeni hormoni (hHG, PrL i Prg) kako bi se pokazale promjene u vježbi. Uzorak bukalnih epitelnih stanica uzet je kod svih muških veslača, prije i poslije vježbe, kao bi se odredio postotak EMN indeksa pomoću unutarstanične mikroelektroforeze. Veća difrerencijacija metaboličkih promjena pokazala se među juniorima nego među seniorima. Ovo se odrazilo promjenama na parametrima ravnoteže na bazi kiseline, na fiziološkim parametrima vježbi, na koncentraciji hormona i promjeni EMN indeksa. Ove promjene vjerojatno su ovisile o dubokim metaboličkim procesima kiselinskog karaktera tijekom vježbanja. Moglo bi se zaključiti da stabilnija homeostaza kroz ekonomičnije metaboličke reakcije u seniora ima utjecaj na proces treniranja, što znači da su seniori bili bolje prilagođeni na teške fizičke vježbe nego juniori.